Nouryon
Nouryon Chemicals LLC

May 14, 2019

US EPA Office of Pollution Prevention and Toxics
1201 Constitution Avenue, NW
WJC East; Room 6428; Attn: Section 8(e)
Washington, DC 20004-3302

SUBJECT: TSCA 8(e) Notice

Dear TSCA Section 8(e) Coordinator:

On behalf of Akzo Nobel Functional Chemicals LLC, a Nouryon Company, we are final submitting results of an OECD 443 Extended One Generation Reproduction Toxicity study conducted on Carbon Disulfide CAS# 75-15-0 as outlined below. The study was commissioned for REACH and sponsored by Carbosulf Chemische Werke GmbH (Acting as the lead company for the TDC-Consortium CS2 which includes the following members: Carbosulf Chemische Werke GmbH, Adisseo France S.A.S.; BASF SE; Lenzing AG; Treuhandgemeinschaft Deutscher; Welding GmbH & Co. KG and AkzoNobel Specialty Chemicals Bv).

Previous letters were submitted to EPA on July 13, 2018 (8EHQ-18-21372), August 10, 2018, October 31, 2018, December 19, 2018, January 24, 2019 and February 21, 2019 containing initial findings. The current submission summarizes the study findings.

The test item and vehicle were administered to Crl: WI(Han) rats once daily by oral Gavage, (0, 1.2, 12, 120 mg/kg/day) 7 days a week. F0-males were treated for a minimum of 12 weeks, including 10 weeks prior to mating and during the mating period, up to and including the day before scheduled necropsy. F0-females were treated for a minimum of 16 weeks, including 10 weeks prior to mating, the variable time to conception, the duration of pregnancy and at least 21 days after delivery, up to and including the day before scheduled necropsy. Females were not dosed during littering.

During lactation (up to PND 21), pups were not treated directly but could potentially be exposed to the test item in utero and/or via maternal milk. From weaning onwards (PND 21), F1-animals of Cohorts 1A, 1B, 1C and 2A were dosed up to and including the day before scheduled necropsy. Cohort 2B was not further dosed and was necropsied at PND 21-22.

F_0-generation

From Week 4 to 5 of treatment onwards, slightly lower mean body weight and body weight gain was observed in male and female rats treated at 120 mg/kg/day, when compared to controls. The difference in mean body weights between controls and the high dose animals gradually increased during the course of treatment. For high dose males, the mean body weight was

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approximately 10% lower at the end of treatment and for high dose females, it was approximately 5% lower at start of mating (after 10 weeks of treatment). During gestation, initially body mean weight gain was comparable for all groups but was again lower in the high dose females from Day 17 post coitum onwards resulting in approximately 10% lower body weights just prior to delivery (Day 20 post coitum) when compared to controls. At the end of lactation, however, the difference in mean body weights between control and high dose females had almost diminished to less than 2% as a result of a significantly higher mean body weight gain in the latter females when compared to control animals. Since the mean food consumption relative to body weight was similar for all groups at all times during treatment, the differences in mean absolute food consumption were considered in line with the changes in body weight gain between the treated animals and controls.

In the hematology parameters, an increase of approximately 25-20% in reticulocyte count, with values outside the historical control range, was observed in both sexes at the end of treatment at 120 mg/kg/day and a dose related increase in white blood cells (WBC) in treated females (i.e. 16%, 26% and 53% higher at 1.2, 12 and 120 mg/kg/day, respectively, when compared to controls). In the high dose females, an increased mean neutrophil/lymphocyte ratio was additionally observed in the differential WBC count (i.e. 0.38 vs 0.63 in control vs high dose). Changes in reticulocyte count could be indicative of changes in red blood cell production and/or destruction, but there were no corroborative changes observed in the other red blood cell parameters and/or histological alterations in the bone marrow. An increased number of white blood cells and neutrophil/lymphocyte ratio could be indicative of (chronic) stress, but there were no corroborative histological changes in the spleen and/or thymus of these high dose females that supported this finding and were therefore considered non-adverse.

The changes in clinical biochemistry parameters comprised increased mean alanine aminotransferase activity in high dose females and a decreased mean potassium at 12 and 120 mg/kg/day and a decreased mean calcium and increased inorganic phosphate at 120 mg/kg in males. The changes in these latter clinical biochemistry parameters were considered of no toxicological relevance, in the absence of any corroborative findings that could be attributed to the changes and not being observed in the opposite sex and in the F_1-animals (see below).

Lower mean serum levels for total thyroxine (T4) were observed in 120 mg/kg/day treated males and females at the end of treatment of 0.51x and 0.69x the levels in controls, respectively. No treatment-related changes in mean thyroid stimulating hormone (TSH) levels and corroborative histopathological alterations in the thyroid gland were apparent in these high dose animals. The mean serum T4 and TSH levels at the end of lactation in F_1-pups of treated females (Cohorts Surplus and 2B) were within the same range as controls.

Given the slight magnitude of these changes (compared to ranges considered normal for rats of this age and strain) and absence of corroborative alterations, all changes in the clinical pathology (i.e. hematology and clinical biochemistry) parameters were considered non-adverse.

Treatment-related changes in organ weights were observed, comprising a lower mean brain weight (absolute) in both sexes, a lower mean thymic weight (absolute and relative) and higher mean liver, adrenal gland and kidney weights (relative) in males, all at 120 mg/kg/day only, and a

lower mean splenic weight (relative) in males at 12 and 120 mg/kg/day. After histopathological examination of each of these organs the following assessment was made:

- The changes in thymic weight correlated to lymphoid depletion observed up to a slight degree in males at 120 mg/kg/day. This was considered non-adverse at the severities noted and in the absence of degenerative changes.
- Microscopic changes were seen in the spleen at 12 and 120 mg/kg/day in males consisting of increased hematopoiesis at 12 and 120 mg/kg/day in males and increased hemosiderin pigment at 120 mg/kg/day in males and females; these were considered non-adverse at the severities noted and in the absence of degenerative changes.
- The changes in liver, kidney and adrenal gland weights in males at 120 mg/kg/day were in line with the decreased final body weight and in the absence of any other indicator of organ toxicity considered not test item-related.
- No histopathological alterations were found in any of the levels in the brain that might explain the decrease in (absolute) brain weight observed in males and females at 120 mg/kg/day. Generally, in F_0-animals the brain weights remain constant over time and are independent from treatment-related changes in body weight. Therefore, the changes in brain weight observed in this study were considered treatment related. Moreover, comparable changes for brain weight were also observed in the F_1-generation (see the discussion below).

Furthermore, microscopic examination of the eyes revealed retinal atrophy affecting the outer nuclear layer in the eye at increased incidence and severity in males and females at 120 mg/kg/day, which was considered to be adverse based on the loss of cell layers in the retina.

In the parameters representative for reproduction, slightly lower mean percentages of motile- and progressive-sperm were observed in 120 mg/kg/day treated males when compared to controls, but these values were within the historical control range. No treatment-related changes were observed in the reproductive parameters in the females up to 120 mg/kg/day.

In the developmental parameters, minimal changes in the post-implantation survival index, litter size and on body and brain weights in F_1-pups were observed.

A slightly lower mean post-implantation survival index was observed in females treated at 12 and 120 mg/kg/day of 88% and 87%, respectively. The low index in females at 12 mg/kg/day was the result of a relatively high number of implantation sites in combination with a normal litter size and was therefore considered an incidental, but normal finding. In females treated at 120 mg/kg/day, however, the difference between the number of implantation sites and pups born (i.e. the post-implantation survival index) was similar to that in females treated at 12 mg/kg/day, but since the mean number of implantation sites was normal in females treated at 120 mg/kg/day, the mean litter sizes in these females were slightly lower when compared to controls. This was supported by the fact that at weaning insufficient pups were available for allocation to all high dose Cohorts.

At birth, the mean body weights of the male and female pups at 120 mg/kg were approximately 5% lower in comparison with controls. The relative difference between control and high dose pup-body weights was still approximately 5% at weaning (PND 21). These results indicated that on average the high dose pups were smaller at birth and remained smaller during lactation in comparison with control (and low and mid dose) animals, but their growth was normal considering their size. This was supported by the lower mean (absolute) brain weights and

similar brain body weight ratios observed in high dose PND 22 pups (Cohort Surplus pups, sacrificed without active dosing at the time of weaning) when compared to control pups. The lower mean high dose pup-body weights at birth probably indicated that their development in utero was slightly retarded when compared to controls.

To further investigate the possible relation of the (minimal) treatment-related effects on reproduction and development observed, i.e. changes in sperm motility and progressivity, post-implantation survival index and litter size and pup weights, it was decided to extend the study with an F_2-generation to be able to clarify any changes.

F_1-generation

At start of treatment of the F_1-generation directly after weaning (PND 21), the difference in mean body weights between the groups was less than 5%. Over the first week of treatment a significantly lower mean body weight gain was observed in both high dose males and females, resulting in respectively 12% and 9% lower mean body weights on Day 8 in comparison with controls. During the remainder of the treatment period the difference in mean body weights between high dose and control males was about constant (i.e. variation between 9-12%). Between Week 1 and 5 of treatment, the high dose females partially recovered from their shortfall in body weight gain over Week 1. From Week 5 onwards, the high dose females showed approximately 5% lower mean body weight in comparison with controls, a difference that was still observed at the start of mating of Cohort 1B females after 10 weeks of treatment (see below). Since the mean food consumption relative to body weight was similar for all groups at all times during treatment, the differences in absolute food consumption were considered in line with the changes in mean body weight between the treated animals and controls.

In the hematology parameters, determined in Cohort 1A animals after 9 weeks of treatment, an increase of approximately 20% in reticulocyte count, with values outside the historical control range, was observed in males at 120 mg/kg/day, but not in females. The increase in mean reticulocyte count in F_1-males was consistent with this finding in F_0-males at the same dose level. The absence of a higher reticulocyte count in high dose F_1-females, as was apparent in F_0-females, might be related to the fact that the F_1-females were nulliparous and the F_0-females were primoparous, and at the end of lactation, and thus might differ in their physiological status. Changes in reticulocyte count could be indicative of changes in red blood cell production and/or destruction, but there were no corroborative changes observed in the other red blood cell parameters and/or histological alterations in the bone marrow in the high dose males.

The changes in clinical biochemistry parameters comprised increased mean aspartate aminotransferase activity and mean level of sodium and chloride and decreased mean level of glucose in high dose males. The changes in these latter clinical biochemistry parameters were considered of no toxicological relevance, in the absence of any corroborative findings that could be attributed to the changes and not being observed in the opposite sex and in the F_0-animals (see above).

Lower mean serum levels for T4 were observed in 120 mg/kg/day treated males at the end of treatment (Cohort 1A) of 0.74x the levels in controls. In 120 mg/kg/day treated females (Cohort

1A), the mean T4 level was almost 10% lower compared to controls. Because the Cohort 1A females were nulliparous and the F_0-females were primoparous, and it is known that pregnancy might affect thyroid hormone levels, T4 levels were also determined in Cohort 1B females at a similar time point after pregnancy as in the F_0-females. Consistent with Cohort 1A females, mean T4 levels in Cohort 1B females were slightly less than 10% lower than in controls, indicating no marked effect on this parameter by pregnancy. No treatment-related changes in TSH and corroborative histopathological alterations were apparent in the thyroid gland in any of these high dose animals. The mean serum T4 and TSH levels at the controls.

Given the slight magnitude of these changes (compared to ranges considered normal for rats of this age and strain) and absence of corroborative alterations, all changes in the clinical pathology parameters (i.e. haematology and clinical biochemistry) were considered non-adverse.

The treatment-related changes in organ weights in Cohort 1A animals were generally consistent with those also observed in F_0-animals and comprising a lower mean brain weight (absolute) in both sexes and a lower mean thymic weight (absolute and relative) in males at 120 mg/kg/day. Upon histopathological examination of each of these organs the following assessment was made:

- Lymphoid depletion was observed up to a slight degree and at a similar incidence in males and females at 120 mg/kg/day. This was considered non-adverse at the severities noted and in the absence of degenerative changes in both sexes. For males this correlated with the decreased mean thymic weight at 120 mg/kg/day.
- No histopathological alterations were found in any levels in the brain that might explain the decrease in (absolute) brain weight observed in males and females at 120 mg/kg/day.

Furthermore, and consistent with the F_0-animals, retinal atrophy of the outer nuclear layer was observed in high dose F_1-males and females up to slight degree. This finding was considered to be adverse based on the loss of cell layers in the retina in animals of both generations.

Cohort 2: Neuro assessment

In Cohort 2 animals, several in-life and/or post-mortem (developmental) neurotoxicity endpoints were assessed. At weaning, the Cohort 2 animals were subdivided into two equal groups, i.e. Cohort 2B, sacrificed without being actively treated before weaning and Cohort 2A sacrificed after at least 8 weeks of treatment.
In the in-life neurotoxic endpoints, foot splay was decreased in Cohort 2A males and females at 120 mg/kg/day (0.65x and 0.75x control, respectively). This finding might be related to the lower mean body weights observed in both sexes, which was considered to be indicative for a smaller body size of the high dose animals in comparison with controls. Moreover, the opposite effect (increased foot splay) would have been expected in case of a developmental neurotoxicant.
No treatment-related effects were observed in the other in-life endpoints in Cohort 2A males and females, i.e. in acoustic startle response, detailed clinical observations, rectal temperature, hearing ability, pupillary reflex, grip strength and motor activity.

In the post-mortem endpoints, changes were observed in brain weight (not only in Cohort 2 animals but in general also in F_0- and F_1-animals) and dimensions of the brain.
Upon morphometric analysis of the brain several differences in linear measurements were

observed, but none of the differences exhibited the same directional trend in males and females or affected animals at both time points. The changes were inconsistent across brain regions, sexes, and time points and several changes were in a direction opposite what would be expected in case of developmental neurotoxicity. Individual animal data were evaluated to investigate a correlation between lower morphometry values and lower brain weight and/or brain gross measurement values, but the correlations were inconsistent. Given these circumstances, the evidence of the test item being a (developmental) neurotoxicant was very limited, if at all. In addition, histopathological examination of the brain did not reveal any treatment-related alterations in F_1 cohort 2A and 2B high dose animals when compared to controls.

From the results obtained from the Cohort 2 animals, it is concluded that there is no evidence for a developmental neurotoxic potential of the test item.

Cohort 1B: Reproductive and developmental results from mating onwards

On the day of mating, the high dose females had approximately 7% lower mean body weights in comparison with the other groups. Over the first two weeks of gestation, mean body weight gain was similar in all groups. Over the third week of gestation reduced mean body weight gain was observed for high dose females resulting in approximately 12% lower mean body weights at the time of delivery, when compared to controls. During lactation, the mean body weight gain in high dose females was higher than in the other groups resulting in a partial recovery of the mean body weight in these females. At termination on lactation Day 21, approximately 5% lower mean body weights were observed in high dose females when compared to controls.

No treatment-related changes were observed in the reproductive parameters in the males up to 120 mg/kg/day. In high dose females, a marked decrease in the mean number of primary and primordial follicles was found of approximately 0.68x that in control females. There was, however, no effect apparent of this change on the mean number of implantation sites or other fertility parameters in these females.

In the developmental parameters, minimal changes in the post-implantation survival index and mean litter size were observed in the high dose group at 120 mg/kg/day. Generally consistent with the changes observed in the F_0-females, a normal number of implantation sites combined with a slightly higher post-implantation survival index resulted in a slightly smaller litter size in high dose F_1-females. Differences in mean body weights of the pups between dose groups were not observed in the F_2-generation either at birth or at the end of lactation (PND 21). However, mean body weight gain in high dose pups over the lactation period was slightly lower (i.e. approximately 10%) in comparison with controls.

Lower mean serum T4 levels were observed in F_0-high dose animals, and to a lesser extent in F_1-high dose animals (Cohort 1A and 1B) at the end of treatment. No changes were found in T4 levels between dose groups in F_1- and F_2-pups at the end of lactation (without being actively treated). Although, the toxicological significance of the changes in T4 levels is unknown, these are in any case not corroborated by treatment-related changes in mean serum levels of TSH and histopathological alterations in the thyroid gland indicating that there was no disruption of thyroid homeostasis in these high dose animals.

Nouryon

Please contact me at (312) 544-7061 if you have any questions regarding this letter.

Sincerely,

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